# DISTRIBUTION OF A RADIOMERCURY-LABELLED DIURETIC (CHLORMERODRIN) IN TISSUES OF MARINE FISH [1]

ROGER L. GREIF AND MARILYN DU VIGNEAUD

*Department of Physiology, Cornell University Medical College, New York City, and the Marine Biological Laboratory, Woods Hole, Mass.*

The ability of mercurial compounds to increase urine flow, recognized at least since the time of Paracelsus (1493–1541), has been widely applied in clinical medicine following the introduction of the organic mercurial diuretics (Saxl and Heilig, 1920). The association of suppression of urine flow with poisoning due to inorganic mercury compounds (Woodman and Tidy, 1877) also suggests that renal tissue is particularly susceptible to the action of such substances. Ludwig and Zillner (1890) were among the early investigators who demonstrated by chemical analysis that the kidneys of dogs poisoned with mercuric chloride contain far higher concentrations of mercury than other tissues. Many subsequent studies, with the use of both biochemical and radioisotopic methods, have confirmed this finding.

$Hg^{203}$-labelled chlormerodrin has recently been used to determine more precisely the location of mercury deposition in mammalian kidney. In dogs and in rats the concentration is highest in the outer renal cortex, decreases in the medulla, and is lowest in renal papillary tissue (Greif *et al.*, 1956). In this connection, Walker and Oliver (1941) have pointed out that the rat outer renal cortex is composed largely of proximal segment of the tubule. The purpose of the present study was to determine, with the use of a radiomercury-labeled organic mercurial diuretic, whether mercury is selectively concentrated in the kidneys of marine fish with a variety of nephron types.

## Materials and Methods

Specimens of flounder (*Paralycthys dentatus*), toadfish (*Opsanus tau*) and dogfish (*Mustelus canis*) were furnished by the Collecting Department of the Marine Biological Laboratory, and were stored briefly in live cars before use. They were weighed and injected with alkaline solutions of chlormerodrin [2] in concentrations indicated in the tables. Most injections were made intramuscularly in the fleshy part of the tail, although a few intramuscular injections were placed immediately dorsal and posterior to the gills. The fish were placed in a running sea water aquarium until sacrificed. At the time of killing, the fish were stunned and bled from either the tail vein or the heart with a needle attached to a heparinized syringe. Tissue was then rapidly removed from the fish, weighed on a torsion balance, and transferred to a test tube for counting in a well-type scintillation counter with scaler. Blocks of tissue were removed from the anterior, medial, and

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posterior portions of the kidney for counting. Results are expressed as milligrams chlormerodrin mercury per gram wet weight of tissue. Unless otherwise indicated, the middle portion of the kidney was selected for comparison with other excised tissues. Urine was obtained either by inserting a pipette into the urogenital papilla or aspirating the exposed bladder with a syringe and needle. Phenol red accumulation in the excised flounder tubule was studied by the method of Forster and Taggart (1950).

## Results

a) *Flounder.* Under the present experimental conditions a high tissue mercury concentration is always found in the kidneys. This is especially striking at the dosage level of one milligram chlormerodrin mercury per kilogram body weight (Table I), an amount which will produce diuresis in mammals (Borghgraef *et al.*, 1956). The larger dose, 10 milligrams mercury per kilogram (Table II), which is toxic to mammals, also proved fatal to some of the fish. Kidney tissue excised from flounder surviving the larger dose was unusually friable. On microscopic examination, the freshly teased tubules appeared cloudy and granular and failed to concentrate phenol red. When such tissue is fixed in Susa fluid and stained with hematoxylin and eosin it cannot be readily distinguished from kidney of fish given

Table I

*Mean tissue concentration of radiomercury administered as one milligram labelled chlormerodrin mercury per kilo body weight. All values expressed as micrograms mercury/gram wet weight of tissue*

| Species | No. of Animals | Plasma | Kidney | Liver | Bile | Gills | Heart | Stomach | Gonads | Spleen |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 2 | 0.7 | 52.2 | 3.2 | 9.7 | 0.2 | 0.5 | 0.4 | 0.2 | 0.7 |
| Toadfish | 1 | 0.8 | 13.2 | 0.3 | 0.0 | 0.5 | 0.0 | 0.5 | 0.0 | 0.2 |
| Dogfish | 1 | 0.9 | 42.2 | 4.4 | 21.9 | 0.0 | 0.0 | 0.3 | 0.8 | 0.5 |
| 24 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 3 | 0.3 | 80.1 | 3.9 | 1.7 | 0.2 | 0.4 | 0.2 | 0.2 | 0.5 |
| Toadfish | 2 | 0.7 | 12.0 | 0.3 | 0.2 | 0.5 | 0.5 | 0.4 | 0.1 | 1.0 |
| Dogfish | 3 | 0.6 | 14.5 | 4.0 | 33.6 | 0.4 | 0.2 | 2.0 | 0.9 | 0.4 |
| 48 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 2 | 3.5 | 35.6 | 6.7 | 2.8 | 1.9 | 0.2 | 0.4 | 0.3 | 1.0 |
| Toadfish | 2 | 0.3 | 9.0 | 0.4 | 0.2 | 0.3 | 0.3 | 0.2 | 1.2 | 0.4 |
| Dogfish | 1 | 0.2 | 6.4 | 2.7 | 21.0 | 0.9 | 0.3 | 2.9 | 0.7 | 0.4 |
| 72 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 2 | 0.1 | 24.6 | 5.7 | 2.7 | 0.2 | 0.2 | 0.0 | 0.2 | 0.7 |
| Dogfish | 1 | 0.1 | 4.4 | 7.3 | 114.0 | 1.0 | 0.2 | 0.4 | 0.7 | 0.4 |

TABLE II

*Mean tissue concentration of radiomercury after administration of 10 milligrams labelled chlormerodrin mercury per kilo body weight. All values expressed as micrograms mercury/gram wet weight of tissue*

| Species | No. of Animals | Plasma | Kidney | Liver | Bile | Gills | Heart | Stomach | Gonads | Spleen |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 4 | 13.6 | 288.0 | 55.6 | 12.8 | 12.5 | 8.2 | 8.7 | 7.3 | 30.2 |
| Toadfish | 2 | 29.5 | 197.0 | 15.0 | 2.6 | 12.3 | 23.5 | 11.7 | 6.4 | 16.5 |
| 24 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 4 | 11.9 | 146.5 | 51.8 | 53.7 | 11.4 | 9.3 | 6.0 | 6.4 | 30.1 |
| Toadfish | 3 | 19.7 | 128.9 | 29.2 | 20.0 | 10.4 | 21.2 | 12.0 | 8.4 | 13.6 |
| 48 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 3 | 12.6 | 77.1 | 59.2 | 121.0 | 10.3 | 8.6 | 8.5 | 9.8 | 37.5 |
| Toadfish | 3 | 19.0 | 105.0 | 36.2 | 37.8 | 11.0 | 21.4 | 10.2 | 5.0 | 16.6 |
| 72 Hours After Intramuscular Injection | | | | | | | | | | |
| Flounder | 2 | 10.2 | 89.4 | 45.1 | 57.0 | 7.1 | 4.2 | 7.7 | 6.7 | 19.6 |
| Toadfish | 3 | 20.8 | 106.5 | 40.9 | 55.7 | 11.7 | 29.2 | 11.5 | 18.5 | 15.8 |

the smaller dose of mercurial or from that of control flounder, although these latter tissues concentrated phenol red and appeared normal when viewed with the dissecting microscope. On the regimen of 10 milligrams Hg per kilogram, high concentrations of mercury are seen not only in the kidney but also in the liver and bile of the surviving animals.

b) *Toadfish.* In Table I and Table II may be found the distribution of chlormerodrin mercury in the tissues of this species. As in the flounder the mercury concentration in the kidney is high, but at 10 milligrams mercury per kilogram the concentration in the bile in toadfish does not exceed the concentration in the kidney. It was possible to obtain blood and urine samples in 11 toadfish after chlormerodrin injection. Despite great individual variation, in all but one animal the urine-to-plasma ratio was at least 4, and the mean value for this ratio was $30.7 \pm 9.6$ (S. E.). Phenol red, injected with the chlormerodrin, also appeared in the urine.

c) *Dogfish.* Tissue mercury distribution in a small number of animals is included in Table I. The most striking difference to be seen in this instance is the high mercury concentration in the bile on the one milligram mercury per kilogram body weight regimen.

*General.* In all animals at the one milligram/kilogram dose the kidney mercury concentration is high, and only in the dogfish is the concentration in the liver and in the bile impressive. It is notable that in none of the animals on any regimen is the mercury concentration in either the gills or the stomach higher than in the

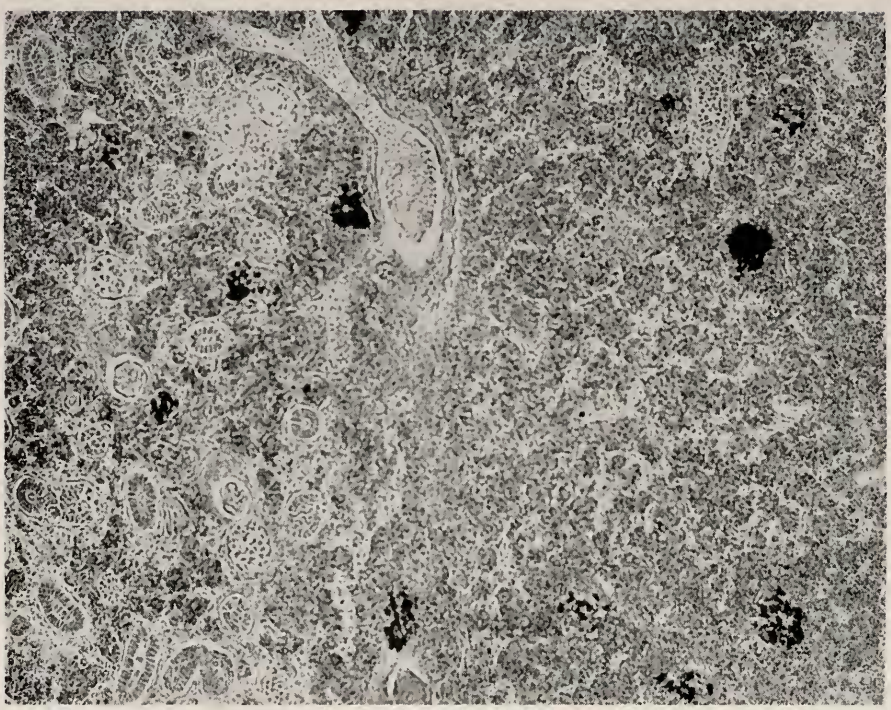

FIGURE 1. Photomicrograph of area of flounder kidney showing transition between anterior portion (right) containing few renal elements, and middle portion in which numerous glomeruli and tubules can be seen.

plasma. The regional distribution of mercury in the kidney is of interest. Both flounder and toadfish kidneys contain much lymphoid tissue in which the renal elements are imbedded, whereas the dogfish kidney on microscopic section shows tubular and glomerular structures surrounded by supporting tissue containing few or no lymphoid elements. Figure 1 shows a photomicrograph of the transition zone

TABLE III

*Regional distribution of radiomercury in fish kidney. Combined data from fish injected with one milligram and 10 milligrams chlormerodrin mercury per kilogram and studied at time intervals up to 72 hours after injection. Results expressed as per cent of mean mercury concentration of tissue from the three regions of the kidney sampled*

| Species | No. of animals | Per cent of total mean mercury concentration in the kidney contributed by: | | |
|---|---|---|---|---|
| | | Anterior sample | Middle sample | Posterior sample |
| Flounder | 16 | 9.0 ± 8.5* | 48.5 ± 5.9 | 42.5 ± 5.6 |
| Toadfish | 15 | 26.6 ± 3.6 | 33.9 ± 5.1 | 39.6 ± 6.5 |
| Dogfish | 6 | 24.9 ± 7.6 | 35.4 ± 3.7 | 39.9 ± 8.2 |

* Standard deviation.

between the anterior and middle section of the flounder kidney. A few renal elements can be seen imbedded in the lymphoid cells in the anterior portion, and these elements can be seen in larger numbers in the more posterior portion of the section. The mercury concentration of the anterior block from which this particular section was made is one fifth that found in the more posterior section.

Table III represents a summary of the regional distribution of radiomercury in the kidneys of the species studied. The mean mercury concentration in anterior, medial, and posterior samples is assigned the value of 100%, and the proportion of the total contributed by the three regions is also expressed as per cent. It can be seen that in each species the lowest mercury concentration is found in the anterior portion, but that in the case of the flounder the difference is particularly striking.

## Discussion

The species of marine vertebrates chosen for this study have nephrons which can be classified roughly into three types. The flounder can be considered to have kidneys with glomerular and proximal segmental function (Forster and Hong, 1958); the dogfish has a complex nephron, with glomerulus, proximal, and distal segment (Kempton, 1943). The toadfish, since the original description by Marshall (1929), has been considered to be both functionally and anatomically an aglomerular species with a nephron consisting of a proximal segment only.

The present experiments show clearly that the presence of a proximal segment is sufficient for a nephron to be capable of concentrating mercury. The high U/P ratio for mercury observed in the toadfish agrees with the findings in dogs of Borghgraef *et al.* (1956) that chlormerodrin mercury is excreted by means of a secretory mechanism. Subsequent "stop-flow" experiments have further localized this mechanism in the dog proximal tubular segment (Kessler *et al.*, 1958). Giebisch and Dorman (1958) have noted the selective accumulation of radiomercury administered as labeled chlormerodrin in the kidneys of carp, chicken, bullfrogs, and turtles, and have shown that in *Necturus maculosus*, a species in which the proximal and distal tubular segments are anatomically distinct, the proximal segment always contains a higher mercury concentration than does the distal. The observation of Bieter (1933) that mercuric chloride induces a diuresis in the anaesthetized toadfish also suggests that mercurial compounds act proximally. Unfortunately no analyses of toadfish urine for sodium and chloride are given with Bieter's experiments. The excretion of phenol red by the toadfish in the present experiments was also observed by Shannon (1938) and others.

The regional distribution of radiomercury noted in Table III deserves some comment. The injections of labelled chlormerodrin were made, for the most part, into the muscular portion of the tail, and since the kidneys of fishes are supplied largely by the renal portal circulation (Smith, 1953), it might be expected that the portion of the kidney closest to the injection site would contain the highest concentration of radiomercury. Such an explanation would agree with the results in both toadfish and dogfish, as seen in Table III, but in the case of the flounder the highest concentration of radioactivity is found in the medial portion of the kidney. It seems probable, therefore, that the low concentration of radiomercury in the anterior portion of the kidney in this species is a reflection of the small number of renal elements imbedded in the lymphoid tissue in this region (Fig. 1) and that

this represents an example of the strikingly higher accumulation of mercury by renal elements than by lymphoid tissue.

Mercurial diuresis in mammals is associated with an increased excretion of sodium and chloride ions. In the case of the marine fish, excretion of the sodium and chloride ions taken in via the mouth appears to be accomplished by the gills (Black, 1957). It is therefore interesting to note that these structures do not accumulate radiomercury under the present experimental conditions. Certain compounds of mercury are concentrated by the mammalian kidney to the same degree as is chlormerodrin mercury without affecting the excretion of water, sodium, or chloride (Kessler *et al.*, 1957). The present experiments with marine fish furnish evidence that the accumulation of mercury against a concentration gradient is a process which may occur in tissues not usually involved in sodium and chloride excretion. Studies in progress with the nephridia of *Phascolosoma gouldi* (Greif, 1957) are in agreement with this evidence.

## Summary

1. Chlormerodrin labelled with radiomercury has been injected into marine fish with different types of nephrons. The kidneys of flounder (*Paralycthys dentatus*), with glomerulus and proximal tubular segment, of dog fish (*Mustelus canis*), with proximal, distal segment, and glomerulus, and toadfish (*Opsanus tau*), with proximal segment only, all concentrate mercury to a high degree.

2. High mercury U/P ratios in the toadfish indicate mercury excretion by a secretory mechanism. Excised flounder tubules will not concentrate phenol red if the animal has previously been injected with a toxic amount of chlormerodrin. Accumulation of mercury is also noted under certain conditions in the bile, especially in the dogfish, but is not seen in either stomach or gills.

3. Evidence is reviewed that in many animal species mercury accumulation is greatest in the proximal tubular segment.

## LITERATURE CITED

Bieter, R. N., 1933. Further studies concerning the action of diuretics upon the aglomerular kidney. *J. Pharmacol. Exper. Therap.*, **49**: 250–256.

Black, V. S., 1957. *In:* The Physiology of Fishes. Editor M. E. Brown, Academic Press, New York, Volume I, p. 188.

Borghgraef, R. R. M., R. H. Kessler and R. F. Pitts, 1956. Plasma regression, distribution and excretion of radiomercury in relation to diuresis following the intravenous administration of $Hg^{203}$- labelled chlormerodrin to the dog. *J. Clin. Invest.*, **35**: 1055–1066.

Forster, R. P., and S. K. Hong, 1958. *In vitro* transport of dyes by isolated renal tubules of the flounder as disclosed by direct visualization. Intracellular accumulation and transcellular movement. *J. Cell. Comp. Physiol.*, **51**: 259–272.

Forster, R. P., and J. V. Taggart, 1950. Use of isolated renal tubules for the examination of metabolic processes associated with active cellular transport. *J. Cell. Comp. Physiol.*, **36**: 251–270.

Giebisch, G., and P. J. Dorman, 1958. Comparative study of uptake and distribution of $Hg^{203}$ given as labelled chlormerodrin. *Proc. Soc. Exper. Biol. Med.*, **98**: 50–52.

Greif, R. L., 1957. Uptake of a radiomercury labeled diuretic (Chlormerodrin) by the nephridia of *Phascolosoma gouldi*. *Biol. Bull.*, **113**: 327.

Greif, R. L., W. J. Sullivan, G. S. Jacobs and R. F. Pitts, 1956. Distribution of radiomercury administered as labelled chlormerodrin in the kidneys of rats and dogs. *J. Clin. Invest.*, **35**: 38–43.

KEMPTON, R. T., 1943. Studies on elasmobranch kidney. I. The structure of the renal tubule of the spiny dogfish *Squalus acanthias*. *J. Morphol.*, **73**: 247–263.

KESSLER, R. H., R. LOZANO AND R. F. PITTS, 1957. Studies on the structure diuretic activity relationships of organic compounds of mercury. *J. Clin. Invest.*, **36**: 656–668.

KESSLER, R. H., K. HIERHOLZER, R. S. GURD AND R. F. PITTS, 1958. Localization of diuretic action of chlormerodrin in the nephron of the dog. *Amer. J. Physiol.*, **194**: 540–546.

LUDWIG, E., AND E. ZILLNER, 1890. Über die Localisation des Quecksilbers im thierischen Organismus nach Vergiftungen mit Aetzsublimat. *Wien. Klin. Wchnschr.*, **3**: 534.

MARSHALL, E. K., 1929. The aglomerular kidney of the toadfish *Opsanus tau*. *Bull. Johns Hopkins Hosp.*, **45**: 95–100.

SAXL, P., AND R. HEILIG, 1920. Über die diuretische Wirkung von Novasurol und anderen Quecksilber-injectionen. *Wien. Klin. Wchnschr.*, **33**: 943–944.

SHANNON, J. A., 1938. The renal excretion of phenol red by the aglomerular fishes *Opsanus tau* and *Lophius piscatorius*. *J. Cell. Comp. Physiol.*, **11**: 315–323.

SMITH, H. W., 1953. From Fish to Philosopher. Little, Brown and Co., Boston, p. 36.

WALKER, A. M., AND J. OLIVER, 1941. Methods for the collection of fluid from single glomeruli and tubules of the mammalian kidney. *Amer. J. Physiol.*, **134**: 562–595.

WOODMAN, W. B., AND C. M. TIDY, 1877. Forensic Medicine and Toxicology. Lindsay and Blakiston, Philadelphia, Pa., p. 210.